

The Claims

The listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Previously Presented) A method for localizing a probe within a cell, the method comprising:

a) providing a sample comprising a cell expressing a membrane bound polypeptide, said polypeptide comprising a single chain antibody that binds to a specific ligand, wherein the ligand is phOx;

b) contacting the sample of a) with a membrane permeant probe/ligand conjugate, the probe/ligand conjugate comprising:

i) a probe moiety,

ii) a ligand comprising phOx, and

iii) a linker moiety coupling the probe to the ligand; and

c) detecting the probe/ligand conjugate within the cell, thereby localizing the probe within the cell.

2. (Previously Presented): The method of claim 1, wherein the probe is a spectroscopic probe.

Claims 3-7 (Canceled)

8. (Previously Presented) The method of claim 1, wherein the polypeptide is a fusion protein.

9. (Previously Presented) The method of claim 1, wherein the detecting comprises NMR imaging.

10. (Previously Presented) The method of claim 1, wherein the detecting comprises positron emission tomography.

11. (Previously Presented) The method of claim 1, wherein the detecting comprises locating the fluorescence characteristic of the fluorescent moiety within the cell.

12. (Previously Presented) The method of claim 1, wherein the detecting comprises fluorescence activated cell sorting.

13. (Previously Presented) The method of claim 1, wherein the cell is an eukaryotic cell.

14. (Previously Presented) The method of claim 1, wherein the cell is a mammalian cell.

15. (Previously Presented) The method of claim 3, further comprising:

- i) adding a stimulus to the cell and
- ii) detecting the probe/ligand conjugate, before and at least one time after addition of the stimulus.

16. (Previously Presented) The method of claim 2, wherein the detecting comprises detecting at least one optical property of the spectroscopic probe.

17. (Previously Presented) The method of claim 16, wherein the optical property is fluorescence emission.

18. (Previously Presented) The method of claim 16, wherein the optical property is fluorescence anisotropy.

Claims 19-59 (Canceled)

60. (Previously Presented) A method for localizing a probe, the method comprising:
- a) providing a sample comprising a cell expressing a specific binding partner, wherein the binding partner is a recombinant membrane bound polypeptide, said polypeptide comprising a single chain antibody that specifically binds to pHx:
 - b) contacting the cell of a) with a probe/ligand conjugate, the probe/ligand conjugate comprising:
 - i) a probe moiety,
 - ii) a ligand comprising pHx, and
 - iii) a linker moiety coupling the probe to the ligand, wherein the ligand and the specific binding partner bind non-covalently, and wherein the probe/ligand conjugate is membrane permeant,
 - c) detecting the probe/ligand conjugate within the cell, thereby localizing the probe within the cell.

Claims 61-63 (Canceled)

64. (Previously Presented) The method of claim 1, wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when it is unbound.

65. (Previously Presented) The method of claim 2, wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when is unbound.

Claims 66-74 (Canceled)

75. (Previously Presented) The method of claim 1, wherein the single chain antibody is bound to a Golgi apparatus membrane or an endoplasmic reticulum membrane.

76. (Canceled)

77. (Previously Presented) The method of claim 1, wherein the single chain antibody comprises:

- a) the amino acid sequence set forth in SEQ ID NO:2;
- b) the amino acid sequence set forth in SEQ ID NO:2 with up to 30 conservative amino acid substitutions;
- c) an amino acid sequence at least 95% identical to SEQ ID NO:2;
- d) an amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:1; or
- e) an amino acid sequence encoded by a nucleic acid sequence at least 95% identical to SEQ ID NO:1.

78. (Previously Presented) The method of claim 1, wherein the linker comprises diaminopentane.

79. (Previously Presented) The method of claim 1, wherein the cell is a living cell.

80. (Previously Presented) The method of claim 1, wherein the probe is a pH sensitive fluorescent probe.

81. (Previously Presented) The method of claim 60, wherein the binding partner is a recombinant membrane bound single chain antibody encoded by:

- a) a nucleic acid sequence comprising SEQ ID NO:1;
- b) a nucleic acid sequence at least 95% identical to SEQ ID NO:1;

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Filed: March 20, 2000
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c) a nucleic acid sequence encoding a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:2 with up to 30 conservative amino acid substitutions;
or

d) a nucleic acid sequence encoding a polypeptide consisting of an amino acid sequence at least 95% identical to SEQ ID NO:2.